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Osmotic and saline effect on growth, water relations, and ion uptake and translocation in *Phlomis purpurea* plants

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The effect of different levels of water deficit and saline stress on physiological and morphological parameters in *Phlomis purpurea* plants was studied to evaluate their adaptability to such conditions. *P. purpurea* plants, grown under greenhouse conditions, were subjected to four irrigation treatments lasting 26 weeks: control (C, 1 dS m^{-1}, 100% water holding capacity), moderate water deficit (MWD, 1dS m^{-1}, 60% of the control level of irrigation water), severe water deficit (SWD, 1 dS m^{-1}, 40% of the control level of irrigation water) and saline (S, 4dS m^{-1}, nutrient solution containing 44 mM NaCl). Aerial dry weight decreased in all three treatments, although this response was more marked in the water deficit treatments, especially SWD. Stem diameter, leaf number and leaf area were similarly reduced in both water deficit treatments, while only leaf area decreased in saline treated plants. Throughout the experiment, plant height remained similar in both control and saline treated plants but was inhibited 10 weeks after the beginning of the deficit irrigation. At the end of the experiment there were significant differences in plant height between the control and saline treatment. The control treatment produced a higher number of plants with flowers. Plants irrigated with saline water had higher Na^+ concentrations in their leaves than in their roots and shoots, while Cl^- concentrations were similar in leaves and roots, suggesting some resistance to

the movement of the latter ions from root to shoots. There was a negative relationship between leaf growth and Na^+ concentration in the saline treated plants, in which the accumulation of salt in leaves was associated with osmotic adjustment, which was responsible for maintaining predawn and midday leaf turgor. However, no osmotic adjustment was observed in plants submitted to water stress. Root hydraulic resistance increased in SWD plants, in which the lowest leaf water potential values were recorded. In water stressed plants, in general the decrease of photosynthesis rate was mainly related with stomata factors, although the reductions observed in saline-stressed plants suggest that non-stomatal limitations to photosynthesis could also have been operating.

Key words: Water stress; Salinity; Gas exchange; Water potential; Osmotic adjustment; Mediterranean shrub.

***Abbreviattions*:** C_i, intercellular CO_2 concentration; EC, electrical conductivity; F_v/F_m, maximal PSII photochemical efficiency; g_s, stomatal conductance; L_p, root hydraulic conductance; P, significance level; P_n, net photosynthesis; Ψ_l, leaf water potential; Ψ_s, leaf osmotic potential; Ψ_t, leaf turgor potential; Ψ_{100s}, leaf osmotic potential at full turgor.

1. Introduction

The use of autochthonous Mediterranean species in xeroscaping, landscaping and revegetation projects has increased in recent years because of their capacity to adapt to stressful environmental conditions. Indeed, many studies have confirmed the ability of Mediterranean plants to adjust their morphology and physiology to environmental stresses (Gulías et al., 2002; Mugnai et al., 2005; Franco et al., 2006). Among other

56 factors, salinity and drought are the major constraints affecting physiological processes
57 and their effects may have severe consequences for plant growth and survival in
58 semiarid regions (Vilagrosa et al., 2003). Therefore, the use of salt and drought-tolerant
59 species for revegetation or xerogardening projects is considered good practice since
60 such plants maintain a normal appearance, despite any water and saline stresses.
61 Although early responses to water and salt stress are very similar, some halophytes can
62 tolerate salt stress, but not drought and some xerophytes can tolerate drought, but not
63 salt stress (Kefu et al., 2003). In this sense, previous research results have indicated that
64 the salt tolerance of ornamental plants varies widely among species and that drought-
65 tolerant native plants are not necessarily salt tolerant. To successfully achieve water
66 conservation in landscaping, research-based information on salt and drought tolerance
67 in landscape plant species with low water requirements is needed.
68 Drought and salt tolerance in plants may be explained by functional and
69 structural adaptations, such as growth regulation, osmotic adjustment, and changes in
70 stomatal conductance and water potential (Zollinger et al., 2007; Sánchez-Blanco et al.,
71 2002), mineral nutrition changes, and hormone balance, all of which may help alleviate
72 the harmful effects of both stresses (Azza Mazher et al., 2007). However, although these
73 mechanisms may allow plants to survive during drought and in saline conditions, they
74 do not necessarily mean that the plants will be of high visual quality. Even plants that
75 have some degree of drought and/or salinity tolerance may show reductions in quality
76 when exposed to stress (Cameron et al., 1999).
77 *Phlomis purpurea* is a member of the Mint family (Lamiaceae). It is a
78 Mediterranean shrub of great interest for ornamental use with its leaves that range from
79 green to grey and flower colour that varies deep purple through pink to white. It shows
80 good adaptability to environmental stresses and can be found in dry and stony habitats,

on roadsides and in field borders. However, little is known about its physiological response to different degrees of drought and water salinity. The purpose of this work was to study the effects on *P. purpurea* plants of the most important abiotic stresses (salinity and drought) which may occur during the nursery phase or in landscaping. Growth, water relations, gas exchange and Na^+ and Cl^- uptake and partitioning between organs were evaluated to ascertain the changes that take place in plants exposed to different levels of drought and salinity and whether these changes confer stress-resistance to the plant. Such knowledge of the salt and drought response of ornamental plants may help the horticultural sector (growers and gardeners) to select species which are more tolerant to salt and/or water stress, while maintaining acceptable appearance.

2. Materials and methods

2.1. Plant material and experimental conditions

Rooted cuttings of *Phlomis purpurea* (Purple phlomis) grown in 5x5x11 cm pots by a specialized nursery were transplanted into 4 L plastic pots (15x15x20 cm) filled with a 5:4:1 (v/v/v) mixture of black peat:coconut fibre:perlite, amended with 2 g L^{-1} of Osmocote Plus (14:13:13 N,P,K plus microelements). Plants were placed in a plastic greenhouse equipped with a cooling system, located in Santomera (Murcia, Spain). The micro-climatic conditions, registered with a Hoboware Lite Data Logger (Escort Data Loggers, Inc., Buchanan, Virginia, USA), were 10 °C (mean minimum), 22 °C (mean maximum) and 15 °C (average) temperature; and 47% (mean minimum), 73% (mean maximum) and 65% (average) relative humidity.

2.2. Experimental design and treatments

After five weeks in the greenhouse, the plants were subjected to four irrigation treatments using a computer-controlled drip irrigation system from November 2007 to May 2008 (26 weeks). The irrigation treatments consisted of a control (C) corresponding to 100% water holding capacity (leaching 15% (v/v) of the applied water), 1 dS m^{-1}; two deficit irrigation treatments: 60% of the control level of irrigation water, 1dS m^{-1} (moderate water deficit; MWD) and 40% of the control irrigation water, 1dS m^{-1} (severe water deficit; SWD) and a saline treatment using tap water with salt added up to 44 mM NaCl (4 dS m^{-1}; S). One drip nozzle delivering 2 L h^{-1} per pot was connected to two spaghetti tubes (one each side of every pot) and the duration of each irrigation episode in the control plants was used to vary the amount of water applied, which depended on the season and on climatic conditions. For the saline treatment, the irrigation water was applied in such a way as to maintain the electrical conductivity of the drainage water at about ± 10% EC of the irrigation water supplied for this treatment. In the control treatment the volume of water varied between 200 and 500 ml per pot and irrigation episode.

In the experiment 42 plants were randomly attributed to each treatment. The data were analysed by one-way ANOVA using Statgraphics Plus for Windows 5.1 software. Ratio and percentage data were subjected to an arcsine square-root transformation before statistical analysis to ensure homogeneity of variance. Treatment means were separated with Duncan's Multiple Range Test ($P \leq 0.05$). Pearson's correlation analysis was used to test for relationship between leaf ion concentrations and leaf dry weight.

2.3. *Growth and mineral concentration*

At the end of the experimental period, the substrate was gently washed from the roots of eight plants per treatment and the plants were divided into shoots (i.e., leaves and stems) and roots. These were then oven-dried at 80 ºC until they reached a constant weight to measure the respective dry weights (DW). Stem diameter (mm), leaf number and leaf area (cm^2) were determined in the same plants, using a leaf area meter (Delta-T; Devices Ltd., Cambridge, UK). Also, the number of plants with flowers was determined in all the plants. Through out the experiment, plant height was measured in 20 plants per treatment.

At the end of the experimental period, eight plants per treatment were harvested and separated into leaves, stems and roots, which were washed with distilled water, dried at 70 ºC and stored at room temperature for inorganic solute analyses. The concentration of Cl^- was analysed by a chloride analyzer (Chloride Analyser Model 926, Sherwood Scientific Ltd.) in the aqueous extracts obtained when mixing 100 mg of dry vegetable powder with 40 ml of water before shaking for 30 min and filtering. The concentrations of Na^+ were determined in a digestion extract with HNO_3:$HClO_4$ (2:1, v/v) by Inductively Coupled Plasma optical emission spectrometer (ICP-OES IRIS INTREPID II XDL).

2.4. Water status and gas exchange

Seasonal changes in leaf water potential (Ψ_l), leaf osmotic potential (Ψ_s) and leaf turgor potential (Ψ_t) at dawn and at midday, leaf osmotic potential at full turgor (Ψ_{100s}), stomatal conductance (g_s), net photosynthesis (P_n) and internal CO_2 concentration (C_i) at midday were determined in five plants per treatment.

Leaf water potential was estimated according to Scholander et al. (1965), using a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA) in which leaves were placed in the chamber within 20 s of collection and pressurised at a rate of 0.02 MPa s^{-1} (Turner, 1988). Leaves from the Ψ_l measurements were frozen in liquid nitrogen (-196 °C) and stored at -30 °C. After thawing, the osmotic potential (Ψ_s) was measured in the extracted sap using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci et al. (1991). Ψ_t was estimated as the difference between leaf water potential (Ψ_l) and leaf osmotic potential (Ψ_s). Leaf osmotic potential at full turgor (Ψ_{100s}) was estimated as indicated above for Ψ_s, using excised leaves with their petioles placed in distilled water overnight to reach full saturation.

Leaf stomatal conductance (g_s), net photosynthetic rate (P_n) and intercellular CO_2 concentration (C_i) were determined in attached leaves using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA). Gas exchange was measured around noon in greenhouse conditions of temperature, light irradiation, CO_2 concentration and relative humidity. During the measurements the values of air temperature were around 20-26 ºC, the CO_2 concentration around 380 μmol mol^{-1}, the relative humidity ranged between 40-60% and pressure deficit was around 1-2 KPa.

Chlorophyll fluorescence on the adaxial leaf surface was measured around noon after illumination with a light intensity of 2500 μmol m^{-2} s^{-1} on eight dark-adapted leaves for 20 min using leafclips (Camejo et al., 2005). The F_v/F_{vm} values were read directly on the fluorometer (OS-30 OptiScience Inc., Tyngsboro, MA, USA). F_v/F_m and the nomenclature used was that of Van Kooten and Snel (1990).

Root hydraulic conductivity (L_p) was determined at the end of the experimental period in eight plants per treatment according to Ramos and Kaufmann (1979). Plants

were de-topped and the substrate was carefully washed from the roots, which were submerged in a container of water and placed in the pressure chamber with the cut stump exposed. The air pressure in the chamber was increased at an approx. rate of 0.4 MPa min^{-1}, up to a final pressure of 0.8 MPa. A small piece of plastic tubing was fitted to the stump and the exudate was collected every 5 min and its volume measured. After the exudation measurements, the root systems were placed in an oven at 80 °C until they reached a constant dry weight. Root hydraulic conductivity was calculated using the formula:

$$L_p = J / (P \times W)$$

where L_p is expressed in mg g^{-1} s^{-1} MPa^{-1}, P is the applied hydrostatic pressure (MPa), W is the dry weight of the root system (in g), and J is the water flow rate through the entire root system (in mg s^{-1}).

3. Results

3.1. Growth analysis and mineral concentration

Salinity and drought stress affected the growth and size of the phlomis plants, and a significant decrease in aerial and root DW and leaf area compared with control plants was measured at the end of the experiment (Table 1). However, the exact effect depended on the treatment and parameter in question. For instance, drought had a more marked effect than salinity, especially SWD on aerial DW. Stem diameter, leaf number and leaf area were similarly reduced in both water deficit treatments, while only leaf decreased in saline plants (Table 1). As regards biomass partitioning with respect to total biomass production, no differences between the control and saline treatment were

observed but higher root /shoot ratios were found in the SWD treatment (Table 1). Throughout the experiment, plant height was similar in the control and saline treatments, but began to be inhibited 10 weeks after application of the deficit irrigation (Fig. 1). Only at the end of the experiment were there significant differences between all the treatments in plant height, the smallest plants (38 cm) being those subjected to severe water stress. The well irrigated plants had the highest number of plants with flowers and the plants of MWD treatment had the lowest number at the end of the experiment (Table 1).

While no accumulation of Cl^- and Na^+ was observed in the plants subjected to water stress (Table 2), the concentrations of both ions increased with salinity. As regard their distribution, Na^+ concentrations were higher in leaves than in roots and shoots, while Cl^- concentration was similar in leaves and roots.

A significant relationship between leaf DW and leaf Na concentration was observed in the saline and control treatments, although there was no significant relationship between leaf DW and Cl^- (Fig. 2A and B).

3.2. Plant water relations and osmotic adjustment

At the end of the experimental period, root hydraulic conductance was lowest in the severe water stressed plants, while no significant differences were observed between saline, MDW and control treatments (Table 1). This was reflected in the seasonal values of the leaf water potential (Ψ_l) at predawn (from -0.25 to -0.44 MPa for the control, from -0.3 to -0.60 MPa for saline and MWD and from -1.2 to -2.0 MPa for the SWD (Fig. 3A). During the experiment, the plants submitted to the MWD and saline treatments had similar Ψ_l values at predawn (before the climatic conditions affected the

232 plant water status), suggesting that both treatments caused a similar level of osmotic
233 stress. When Ψ_l was determined at midday, the SWD treatment showed the lowest
234 values, around -3.0 MPa (Fig. 3B).
235 Leaf turgor potential (Ψ_t) decreased in the water stress treatments, especially in
236 SWD (Fig. 3C, D). No differences in Ψ_{100s} between the water stress treatments and the
237 control were found during the experimental period (Fig. 4), pointing to an absence of
238 osmotic adjustment in these treatments. While there was a tendency for Ψ_{100s} to
239 decrease in the saline treatment, differences were only significant at the end of the
240 experimental period.
241
242 *3.3. Stomatal conductance and photosynthetic parameters*
243
244 The plants subjected to water stress showed lower stomatal conductance values
245 than the control from the beginning of the experiment, especially in the case of severe
246 water stress (Fig. 5A). Such reductions with respect to the control plants were also
247 observed in the photosynthesis levels in both water stress treatments, although the
248 differences were less pronounced (Fig. 5B). The g_s values fell later in the saline than in
249 the water stress treatments and the effect on P_n was stronger than in the water stressed
250 plants at the end of the experiment. Both water stress treatments showed the higher $P_n/$
251 g_s ratios (intrinsic water use efficiency) and higher photosynthetic rates when stomatal
252 opening was reduced (Fig 5C). MWD plants were able to maintain P_n values similar to
253 control values when stomatal conductance decreased by 40% and SWD maintained
254 acceptable photosynthetic rates when stomatal opening was about 20% of the control
255 values.

In water stressed plants, the internal CO_2 concentration, C_i, behaved similarly to g_s and P_n throughout the experiment (Fig.5D), although at the end of the experimental period the reductions in C_i with respect to the control were greater than the reductions observed in the photosynthesis rate. C_i also decreased in saline treatment, although this response was less marked than the decrease in g_s and P_n values.

After 26 weeks under stress conditions the reduction in g_s was 1.5 times the reduction in C_i in both water stressed plants, while in salt stressed plants the corresponding reduction in g_s was three times that of C_i (all measured with respect to the control values).

No significant changes were observed in the chlorophyll fluorescence (F_v/F_m) values, which remained at around 0.70-0.75 in all treatments (Table 1).

4. Discussion

Ornamental shrubs in general have demonstrated wide variability in their reaction to water stress and salinity (Cassaniti et al., 2009). The responses of plant species to salinity and osmotic stress in terms of growth are the ultimate expression of several interacting physiological and biochemical parameters (Sidari et al., 2008). In our conditions, both soil drying and salinity reduced plant growth, especially in drought-exposed plants, which showed the lowest stem and leaf dry weight values, resulting in an increased root/shoot dry weight ratio. This latter response was not maintained when plants were exposed to salinity. The different distribution of biomass induced by both stress situations may be due to the need to maintain a higher root surface area under drought conditions and the need to reduce root volume in plants exposed to salinity,

280 which may be a favourable trait limiting their capacity to accumulate toxic ions in the
281 shoot (Munns, 2002; Alarcón et al., 2006).
282 The effect of salt stress and water stress on plant growth and dry matter
283 accumulation has been described in several crops species (Shannon and Grieve, 1999;
284 Sánchez-Blanco et al., 2002; Rodriguez et al., 2005). These stresses have been used to
285 modulate the height and shape of ornamental shrubs and to control plant growth without
286 losing the original ornamental characteristics (Pardossi and Vernieri, 2002; Cameron et
287 al., 2006; Álvarez et al., 2009).
288 The different water stress levels applied to phlomis plants in our experiment
289 induced different growth responses, as was also reported by Alarcón et al. (2006) in
290 *Rosmarinus officinalis*. The water stress level must be considered an important aspect
291 when water stress is used as a technique for reducing plant size in ornamental plants
292 (Vernieri et al., 2006).
293 Reductions in leaf canopy surface have been considered as an avoidance
294 mechanism which minimises water loss under stress conditions (Savé et al., 1994).
295 Also, water deficit may promote floral initiation as a strategy to maintain reproductive
296 capacity in adverse conditions. However, there also are reports that floral initiation can
297 be inhibited by water deficits (Sharp et al., 2009). In our case, both water deficit
298 treatments reduced the percentage of flowering plants at the end of the experiment. This
299 was particularly true in the case of MWD, although the same treatment produced a
300 higher number of plants with flowers than control at an earlier stage (data not shown).
301 Maybe the time to promote flowering was different and depend on the level of the water
302 stress applied.
303 Moreover, the effects of deficit irrigation on flowering depend on the floral
304 index chosen to express the flowering data (Sharp et al., 2009). The MWD treatment

305 resulted in a reduction in the number of plants with flowers al the end of the experiment,
306 but the time to initiation was shorter. A response that may be important for judging
307 plant quality.

308 As regards the saline treatment, it is well known that salinity reduces the
309 vegetative and reproductive growth of non-halophytes (Azza Mazher et al., 2007).
310 Purple phlomis could be considered a moderately salt tolerant species as it showed little
311 growth reduction (28%) and few symptoms of leaf injury, both factors that have been
312 used as a measure of resistance to saline conditions (Sánchez-Blanco et al., 1991; Bañón
313 et al., 2005).

314 An increase in external NaCl concentrations induces an increase of Na^+ and Cl^-
315 in roots and leaves of different ornamental species (Cassaniti et al., 2009). Phlomis
316 plants submitted to salt treatment increased their Na^+ concentration, especially in leaves,
317 while the increase in the Cl^- concentration was similarly for roots and leaves. An
318 analogous response was recorded for Bougainvillea (Cassaniti et al., 2009). Some
319 species show a special ability to differentiate between $Na^+$and Cl^-, which has been
320 related to salt secretion through glandular hairs (Remadan and Flowers, 2004). Chloride
321 has been described to be more toxic than Na^+ when it accumulates in excess in leaves
322 (Fornes et al., 2007).

323 The accumulation of Na^+ and Cl^- in leaves has been correlated with growth
324 reductions (García-Legaz et al., 2005). In our conditions the plants irrigated with saline
325 water of 4 dS m^{-1} EC showed a lower degree of growth reduction than those subjected to
326 water stress, which reflects the tolerance of this species to this level of salinity.

327 In some cases tolerance has been related to higher ion concentrations in the roots
328 compared with the leaves (Boursier and Lauchli, 1990), which would suggest a limited
329 transport to the shoots (Colmer et al., 2005). Our results for *P. purpurea* did not confirm

this finding, especially in the case of the Na^+ ions, although the plants did show an ability to differentiate between Na^+ and Cl^- retention and transport, as other authors have verified (Romero et al., 1997). Whatever the case, the accumulation of Na^+ and Cl^- in plant tissues did not induce any symptoms of necrosis, suggesting a certain degree of salt compartmentalisation and exclusion from the cytoplasm (Sánchez-Blanco et al., 2004; Rodríguez et al., 2005).

As far as plant water status is concerned, both predawn and midday leaf water potential values indicate that severe water stress caused a pronounced dehydration throughout the experiment. This would be due to difficulty in taking up water from the substrate, as can be seen from the values recorded for root hydraulic resistance. An increase in the resistance to water flow from soil to plant in drought conditions had been observed in many species (De Herralde et al., 1998; Sánchez-Blanco et al., 2002). In general, salinity led a drop in Ψ_l, similar to that found in MWD at predawn, suggesting that both treatments provided similar available substrate water content (Choné et al., 2001).

The observed decrease in leaf osmotic potential at full turgor in salinized plants underlines the osmotic adjustment process that takes place under these conditions. Such osmotic adjustment permitted the turgor potential to be maintained in saline-treated plants throughout the experiment in spite of a moderate osmotic adjustment (0.2 MPa). This behaviour and the values of osmotic adjustment observed are within those reported for other studies on Mediterranean ornamental plants submitted to saline stress (Sánchez-Blanco et al., 1998; Rodríguez et al., 2005; Navarro et al., 2008). *P. purpurea* may behave as a typical Na^+ includer, compartmentalizing Na^+ and Cl^- within the leaf cell vacuoles, where it may be used as osmoticum to lower the osmotic potential

354 necessary for the maintenance of leaf turgor (Koyro et al., 2006). Osmotic adjustment
355 was not observed in plants subjected to drought.
356 This could indicate that these solutes were responsible for the osmotic
357 adjustment described above. In relation to the comparative physiology of osmotic
358 adjustment in saline versus dry soil, the issue of metabolic costs should be considered.
359 The cost of intracellular compartmentation is relatively small compared with that
360 needed to synthesize organic solutes for osmotic adjustment (Munns, 2002).
361 The reduction in P_n in water stressed plants was related with a lower g_s, although
362 photosynthesis activity remained high in spite of the reduced stomatal conductance.
363 This indicates an increase in intrinsic water use efficiency (P_n/ g_s), a response that was
364 more marked in SWD plants, as has been observed in *Callistemon* plants (Vernieri et
365 al., 2006). The close association between P_n, g_s and C_i in water stressed plants suggests
366 that a decline in net photosynthesis is largely a consequence of stomatal limitation
367 (Bacelar et al., 2007).
368 According to Colom and Vazzana (2003) decreases in P_n under drought
369 conditions are related with the reduced stomatal opening imposed by the water deficit,
370 although this observation does not exclude the possibility of metabolic damage due to
371 water deficit when the drought severity increases and environmental conditions become
372 more stressful.
373 At the end of the experimental growing period, photosynthesis was seen to be
374 more negatively affected in plants subjected to salinity, which could be related to the
375 high concentration of Cl^- and Na^+ accumulated in leaves. In these plants, both P_n and g_s
376 were reduced in a similar way, In most saline situations, photosynthesis is depressed
377 due to reductions in stomatal and mesophyll conductance to CO_2 (Flexas et al., 2004).

Koyro et al. (2006) suggest that reductions in stomatal conductance represent adaptative mechanisms to cope with excessive salt, reducing the salt load of leaves and helping to increase longevity by maintaining salts at subtoxic levels for longer than would occur if transpiration rates were not diminished.

The impact of salinity on photosynthetic parameters has already been reported (Tattini and Traversi, 2008; Mugnai et al., 2009). Reduced net CO_2 assimilation rates with increasing salinity have been attributed to (i) stomatal closure, leading to a reduction in intracellular CO_2 partial pressure, (ii) concurrent non-stomatal factors (i.e., the reduction in protein concentration), (iii) a decline in photosynthetic pigments and (iv) changes in ion concentrations. As mentioned above, salinity had a negative effect on the photosynthetic rate and stomatal conductance of purple phlomis plants but the intercellular CO_2 was much less affected. Similar results were obtained by Bacelar et al. (2007) in plants subjected to severe drought stress. This indicates that the reduction in photosynthetic rate observed did not simply depend on stomatal closure and the elimination of intercellular CO_2, but probably on non-stomatal factors as well. The transition from stomatal to biochemical limitations could be one possible mechanism to account for the decrease in photosynthetic rate following saline stress in this study (Bolla et al., 2010). According to Flexas et al. (2009) and Galle et al. (2009), moderate or short term stress effects are mainly stomatal, whereas severe or prolonged stress reduces the capacity of CO_2 fixation and increase mesophyll resistance.

In the saline treatment, the inhibition of photosynthesis observed at the end of the experiment was reflected in the inhibition of photo-assimilation and dry matter production (height decrease) even through leaf turgor was maintained at a similar level to control plants throughout the experiment. Whatever the case, such osmotic

adjustment has been well documented as an adaptation to drought and salinity stress (Morgan, 1984).

As regards fluorescence, the values did not change with respect to controls in either the water or salt stress scenarios, and so no damage occurred to the photosynthetic system. Many studies use a sustained decrease in the maximum efficiency of PSII in dark-adapted leaves (F_v/F_m) as a reliable diagnosis of photoinhibition in response to different stresses. It has been suggested that F_v/F_m below 0.83 be usually reflect stressed plants (Maxwell and Johnson, 2000). However, other researchers have suggested that some species can not be considered stressed until much lower F_v/F_m values are reached (Colom and Vazzana, 2003; Percival, 2006; Bacelar et al., 2007).

In conclusion, our results indicate that although reduced irrigation and the use of saline water could be useful for controlling plant size in *Phlomis purpurea* for use as a flowering pot plant, the morphological and physiological responses differ significantly between salinity and water stress. Severe water stress induced an excessive decrease in plant height and growth due to leaf tissue dehydration, causing stomatal closure and a decrease in CO_2 absorption. In the moderate water stress applied, most of these responses were mitigated. Salt may improve the response of *P. purpurea* to water stress as a result of a slight growth reduction and the absence of toxicity symptoms related to osmotic adjustment. It can be inferred that the use of saline water (around 4 dS m^{-1}) is feasible for growing this ornamental plant commercially, a consideration that is particularly relevant in arid saline areas.

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References

Alarcón, J.J., Morales, M.A., Ferrández, T., Sánchez-Blanco, M.J., 2006. Effects of water and salt stresses on growth, water relations and gas exchange in *Rosmarinus officinalis*. J. Hor. Sci. Biotehcnol. 81, 845-853.

Álvarez, S., Navarro, A., Bañón, S., Sánchez-Blanco, M.J., 2009. Regulated deficit irrigation in potted *dianthus* plants: effects of severe and moderate water stress on growth and physiological responses. Sci. Hort. 122, 579-585.

Azza Mazher, A.M., Fatma El-Quesni, E.M., Farahat, M.M., 2007. Responses of ornamental plants and woody trees to salinity. World J. Agric. Sci. 3, 386-395.

Bacelar, E.A., Santos, D.L., Moutinho-Pereira, J.M., Lopes, J.L., Gonçalves, B.C., Ferreira, T.C., Correia, C.M. 2007. Physiological behaviour, oxidative damage and antioxidative protection of olive trees grown under different irrigation regimes. Plant Soil 292, 1-12.

Bañon, S., Ochoa, J., Fernández, J.A., Sánchez-Blanco, M.J. 2005. Paclobutrazol as an aid to reducing some effects of salt stress oleander seedlings. Eur. J. Hort. Sci. 70, 43-49.

Boursier, P., Läuchli, A., 1990. Growth responses and mineral nutrient relations of salt stressed sorghum. Crop Sci. 30, 1226-1233.

Camejo, D., Rodríguez, P., Morales, M.A., Dell'Amico, J., Torrecillas, A, Alarcón, J.J., 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. J. Plant Physiol. 162, 281–289.

451 Cameron, R.W.F., Harrison-Murray, R.S., Scott, M.A., 1999. The use of controlled
452 water stress to manipulate growth of container-grown *Rhododendron* cv. Hoppy.
453 J. Hort. Sci. Biotechnol. 74,161–169.

454 Cameron, R.W.F., Harrison-Murray, R.S., Atkinson, C.J., Judd, H.L., 2006. Regulated
455 deficit irrigation: a means to control growth in woody ornamentals. J. Hort. Sci.
456 Biotechnol. 81, 435-443.

457 Cassaniti, C, Leonardi, C, Flowers, T., 2009. The effects of sodium chloride on
458 ornamental shrubs. Sci. Hort. 122, 586-593.

459 Choné, X., Van Leeuwen, C., Dubourdieu, D., Gaudillère, J.P. 2001. Stem water
460 potential is a sensitive indicator of Grapevine Water status. Ann. Bot. 87, 477-
461 483.

462 Colmer, T.D., Muñiz, R., Flowers, T.J., 2005. Improving salt tolerance of wheat and
463 barley: future prospects. Aust. J. Exp. Agric. 45, 1425-1443.

464 Colom, M.R., Vazzana, C. 2003. Photosynthesis and PSII functionality of drought-
465 resistant and drought-sensitive weeping lovegrass plants. Environ. Exp. Bot. 49,
466 135-144.

467 De Herralde**,** F., Biel C., Savé, R., Morales, M.A., Torrecillas, A., Alarcón, J.J.,
468 Sánchez-Blanco, M.J., 1998. Effect of water and salt stresses on the growth, gas
469 exchange and water relations in *Argyranthemum coronopifolium* plants. Plant Sci.
470 139, 9-17.

471 Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T.D., 2004. Diffusive and
472 metabolic limitations to photosynthesis under drought and salinity in C3 plants.
473 Plant Biol. 6, 269-279.

Flexas, J., Barón, M., Bota, J., Ducruet, J. M., Gallé, A., Galmes, J., Jiménez, M., Pou, A., Ribas-Carbó, M., Sajnani, C., Tomàs, M., Medrano, H.. 2009. Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted *Vitis* hybrid Ritcher-110 (*V. berlandie*ri×*V. rupestris*). J. Exp. Bot. 60, 2361-2377.

Fornes, F., Belda, R.M,, Carrión, C., Noguera, V., García-Agustín, P., Abad, M. 2007. Pre-conditioning ornamental plants to drought by means of saline water irrigation as related to salinity tolerance. Sci. Hort. 113, 52-59

Franco, J.A., Martínez-Sánchez, J.J., Fernández, J.A., Bañón, S., 2006. Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. J. Hort. Sci. Biotechnol. 81, 3-17

Gallé, A., Flores-Sarasa, I., Tomas, M., Pou, A., Medrano, H., Ribas-Carbó, M., Flexas, J. 2009. The role of mesophyll conductance during water stress and recovery in tobacco (*Nicotina sylvestris* L.) acclimation or limitation. J. Exp. Bot. 60, 2379-2390.

García-Legaz, M.F., López-Gómez, E., Mataix Beneyto, J., Torrecillas, A., Sánchez-Blanco, M.J., 2005. Effects of salinity and rootstock on growth, water relations, nutrition and gas exchange of loquat. J. Hort. Sci. Biotechnol. 80, 199-203.

Gucci, R., Xiloyannis, C., Flore, J.A., 1991. Gas exchange parameters water relations and carbohydrate partitioning in leaves of field-grown *Prunus domestica* following fruit removal. Physiol. Plant. 83, 497-505.

Gulías, J. Flexas, J., Abadía, A., Medrano, H., 2002. Photosynthetic response to water deficit in six Mediterranean sclerophyll species: possible factors explaining the declining distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species. Tree Physiol. 22, 687–697

499 Kefu, Z., Hai, F., San, Z., Jie, S., 2003. Study on the salt and drought tolerance of
500 *Suaeda salsa* and *Kalanchoe claigremontiana* under isoosmotic salt and water
501 stress. Plant Sci. 165, 837-844.

502 Koyro, H. W. 2006. Effect of salinity of growth, photosynthesis, water relations and
503 solute composition of the potential cash crop halophyte *Plantago coronopus* (L.)
504 Environ. Exp. Bot. 56, 136-146.

505 Maxwell, K., Johnson, G.N. 2000. Chlorophyll fluorescence: A practical guide. J. Exp.
506 Bot. 51,659-668.

507 Morgan, J.M., 1984. Osmoregulation and water stress in higher plants. Annu. Rev. Plant
508 Physiol. 35, 299-319.

509 Mugnai, S., Vernieri, P., Malorgio, F., Serra, G., 2005. Response of some ornamental
510 shrubs to different soil water conditions. Adv. Hort. Sci. 19, 94-100.

511 Mugnai, S., Ferrante, A., Petrognani, L., Serra, G., Vernieri, P. 2009. Stres-Induced
512 Variation in Leaf Gas Exchange and Chlorophyll a Fluorescence in *Callistemon*
513 Plants. Res. J. Biol. Sci. 4, 913-921.

514 Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ.
515 25, 239-250.

516 Navarro, A., Bañón, S., Conejero, W., Sánchez-Blanco, M.J. 2008. Ornamental
517 characters, ion accumulation and water status in *Arbutus unedo* seedlings irrigated
518 with saline water and subsequent relief and transplanting. Environ. Exp. Bot. 62,
519 364-370.

520 Pardossi, A., Vernieri, P., 2002. Metodi non convenzionali per il controllo della taglia
521 delle piante ornamentali cultivate in serra. Inform. Agr. 23, 41-47.

522 Percival, G.C., Kearby, I.P., Al-Habsib, S. 2006. An assessment of the drought
523 tolerance of *Fraxinus* genotypes for urban landscaping plantings. Urban For
524 Urban Green 5, 17-27.

525 Ramos, C., Kaufman, M.R. 1979. Hydraulic resistance of rouge lemon roots. Physiol.
526 Plantarum 45, 311-314.

527 Ramadan, T., Flower, T.J. 2004. Effects of salinity and benzyl adenine on
528 development and function of microhairs of *Zea mays* L. Planta 219, 639-648.

529 Rodríguez, P., Torrecillas, A., Morales, M.A., Ortuño, M.F., Sánchez-Blanco, M.J.,
530 2005. Effects of NaCl salinity and water stress on growth and leaf water relations
531 of *Asteriscus maritimus* plants. Environ. Exp. Bot. 53, 113-123

532 Romero, L., Belakbir, A., Ragala, L., Ruiz, J.M., 1997.Response of plant yield and leaf
533 pigments to saline conditions: effectiveness of different rootstocks in melon plants
534 (*Cucumis melo* L.). Soil Sci. Plant Nutr.. 43,855-862.

535 Sánchez-Blanco, M.J., Bolarín, M.C., Morales, M.A., Alarcón, J.J., Torrecillas, A.,
536 1991. Salinity effect on water relations in *Lycopersicum esculentum* and its wild
537 salt-tolerance relative species, *L. pennellii*. Physiol. Plant. 83, 269-274.

538 Sánchez-Blanco, M.J., Morales, M.A., Torrecillas, A., Alarcón, J.J., 1998. Diurnal and
539 seasonal osmotic potencial changes in *Lotus creticus* plants grown under saline
540 stress. Plant Sci. 136, 1-10.

541 Sánchez-Blanco, M.J., Rodríguez, P., Morales, M. A., Ortuño, M. F., Torrecillas, A.,
542 2002. Comparative growth and water relation of *Cistus albidus* and *Cistus*
543 *monspeliensis* plants during water deficit conditions and recovery. Plant Sci. 162,
544 107-113

545 Sánchez-Blanco, M.J., Ferrández, T., Navarro, A., Bañón, S., Alarcón, J.J., 2004.
546 Effects of irrigation and air humidity preconditioning on water relations, growth
547 and survival of *Rosmarinus officinalis* plants during and after transplanting. J.
548 Plant Physiol. 161, 1133–1142.

549 Savé, R., Olivella, C., Biel, C., Adillón, J., Rabella, R., 1994. Seasonal patterns of water
550 relationships, photosynthetic pigments and morphology of *Actinidia deliciosa*
551 plants of the Haywards and Tomouri cultivars. Agronomie 2, 121–126

552 Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemingsen, E.A., 1965. Sap pressure
553 in vascular plants. Scientia 148,339-346.

554 Shannon, M.C., Grieve, C.M., 1999. Tolerance of vegetable crops to salinity. Sci. Hort.
555 78, 5-38.

556 Sharp, R.G., Else, M.A., Cameron, R.G., Davies, W.J. 2009. Water deficits promote
557 flowering in *Rhododendron* via regulation of pre and post initiation development.
558 Sci. Hort. 120, 511-517.

559 Sidari, M., Mallamaci, C., Muscolo, M., 2008. Drought, salinity and heat differently
560 affect seed germination of *Pinus pinea*. J. Forest Res. 13, 326-330.

561 Tattini, M., Traversi, M.L. 2008. On the mechanism of salt tolerance in olive (*Olea*
562 *europaea* L.) under low or high Ca^{2+} supply. Environ. Exp. Bot. 65, 72-81.

563 Turner, N.C., 1988. Measurement of plant water status by the pressure chamber
564 technique. Irrig. Sci. 9, 289-308.

565 Van Kooten, O., Snel, F.H., 1990. The use of chlorophyll fluorescence nomenclature in
566 plant stress physiology. Photosynth. Res. 25, 147-150.

567 Vernieri, P., Mugnai, S., Borghesi, E., Petrognani, L. Serra, G., 2006. Non-chemical
568 growth control of potted *Callistemon laevis*. Agric. Med. 160, 85-90.
569 Vilagrosa, A., Bellot, J., Vallejo, V.R., Gil-Pelegrín, E., 2003. Cavitation, stomatal
570 conductance, and leaf dieback in seedlings of two co-ocurring Mediterranean
571 shrubs during an intense drought, J. Exp. Bot. 54, 2015-2024.
572 Zollinger, N., Koenig, R., Cerny-Koenig, T., Kjelgren, R., 2007. Relative salinity
573 tolerance of Intermountain Western United States native herbaceous perennials.
574 HortScience 42, 529-534.
575

Tables

Table 1

Growth and biomass traits and root hydraulic conductance (L_p) at the end of the experiment in *P. purpurea* plants subjected to different irrigation treatments. Values are the mean of eight plants, except in the case "plants with flowers", when the values are the mean of two entire rows of growing plants.

Parameters	Treatments C	S	MWD	SWD	P
Aerial DW (g plant^{-1})	32.42 ± 1.75 a	22.95 ± 1.40 b	16.34 ± 0.70 c	11.09 ± 0.60 d	***
Root DW (g plant^{-1})	24.60 ± 2.09 a	17.83 ± 1.05 b	16.63 ± 0.76 b	15.37 ± 0.70 b	***
Total leaf area (cm^2)	2084 ± 112 a	1639 ± 103 b	851 ± 66 c	658 ± 57 c	***
Stem diameter (mm)	6.31 ± 0.35 a	5.95 ± 0.29 a	4.56 ± 0.22 b	4.50 ± 0.22 b	***
Root/Shoot ratio	0.79 ± 0.10 b	0.79 ± 0.06 b	1.03 ± 0.05 b	1.42 ± 0.11 a	***
Leaf number	160.2 ± 6.6 a	145.7 ± 11.4 a	109.8 ± 5.4 b	112.4 ± 6.8 b	***
Plants with flowers (%)	46.67 ± 3.2 a	30 ± 1.6 b	13.3 ± 1.2 c	34.03 ± 3.0 b	**
L_p(mg s^{-1}MPa^{-1} g^{-1})	0.41 ± 0.01 a	0.43 ± 0.03 a	0.41 ± 0.01 a	0.26 ± 0.01 b	***
F_v/F_m	0.749 ± 0.022	0.707 ± 0.015	0.717 ± 0.022	0.735 ± 0.016	ns

Means within a row without a common letter are significantly different by Duncan $_{0.05}$ test.

P: probability level

ns: not significant

** P ≤0.1.

*** P ≤ 0.001.

Table 2

Na^+ and Cl^- concentrations at the end of the experiment in *P. purpurea* plants subjected to different irrigation treatments. Values are the mean of eight plants.

(mmol g^{-1} DW)		Treatments C	S	MWD	SWD	P
Na^+	Leaves	0.249 ± 0.034 b	1.158 ± 0.116 aC	0.325 ± 0.016 b	0.328 ± 0.023 b	***
	Stem	0.162 ± 0.020 b	0.351 ± 0.035 aE	0.099 ± 0.006 b	0.100 ± 0.010 b	***
	Root	0.321 ± 0.020 b	0.617 ± 0.024 aD	0.224 ± 0.016 c	0.286 ± 0.015 b	***
Cl^-	Leaves	0.306 ± 0.028 b	0.794 ± 0.199 aA	0.393 ± 0.026 b	0.425 ± 0.254 b	*
	Stem	0.251 ± 0.042	0.334 ± 0.054 B	0.245 ± 0.026	0.225 ± 0.038	ns
	Root	0.251 ± 0.030 b	0.875 ± 0.165 aA	0.273 ± 0.025 b	0.245 ± 0.025 b	***

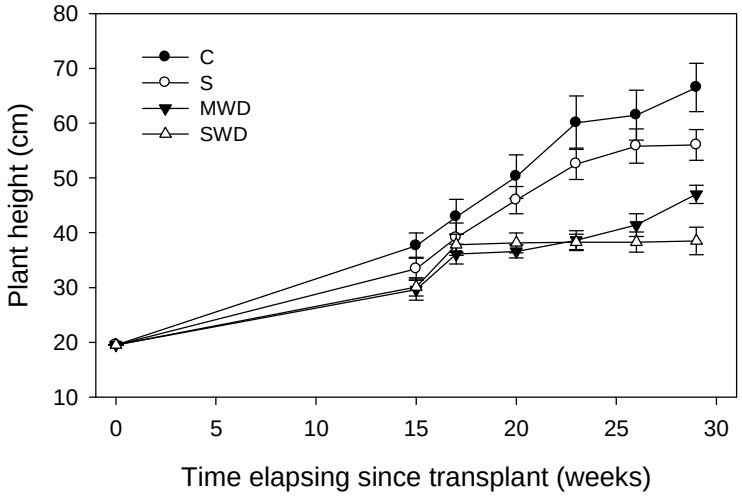
Means within a row without a common lowercase letter are significantly different by Duncan $_{0.05}$ test. Means within a column without a common capital letter are significantly different by Duncan $_{0.05}$ test.

P: probability level

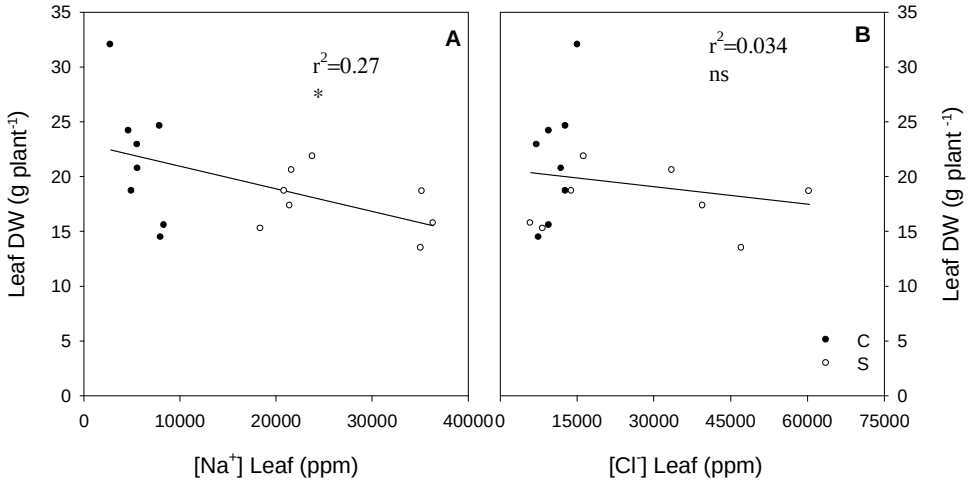
* P ≤ 0.05.

*** P ≤ 0.001.

603 **Figures**



604
605 **Fig. 1.** Height of *P. purpurea* plants subjected to different irrigation treatments. Values
606 are means of 20 plants per treatment and the vertical bars indicate standard errors.



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608 **Fig. 2.** Relationship between leaf DW and Na^+ (A) and Cl^- (B) leaf concentrations at the
609 end of the experiment in *P. purpurea* plants subjected to control and saline treatment.

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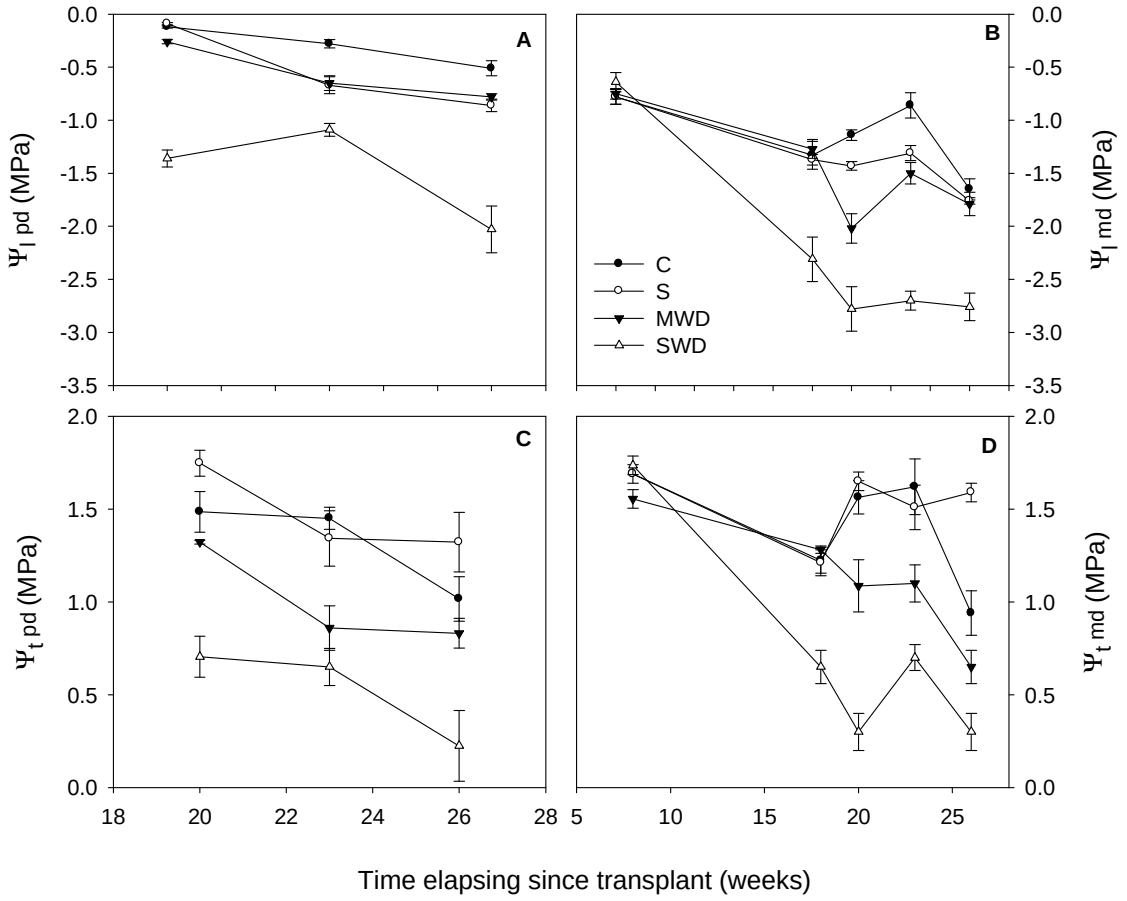
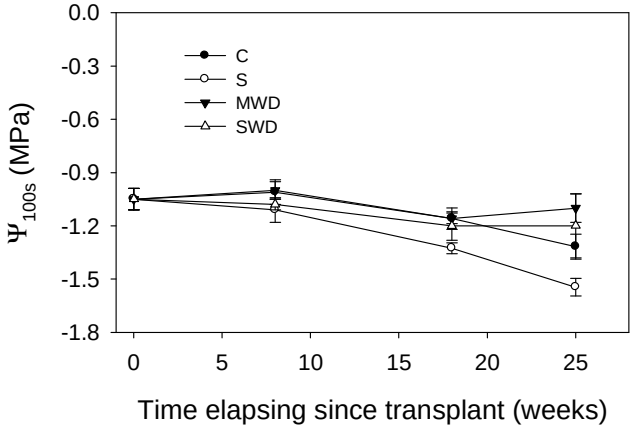


611

612 **Fig. 3.** Evolution of leaf water potential at pre-dawn ($\Psi_{l\ pd}$, A) and midday ($\Psi_{l\ md}$, B),
613 leaf turgor potential at predawn ($\Psi_{t\ pd}$, C) and midday ($\Psi_{t\ md}$, D) in *P. purpurea* plants
614 subjected to different irrigation treatments. Values are means of five plants per
615 treatment and the vertical bars indicate standard errors.



616

617 **Fig. 4**. Leaf osmotic potential at full turgor at midday (Ψ_{100s}) in *P. purpurea* plants
618 subjected to different irrigation treatments. Values are means of five plants per
619 treatment and the vertical bars indicate standard errors.
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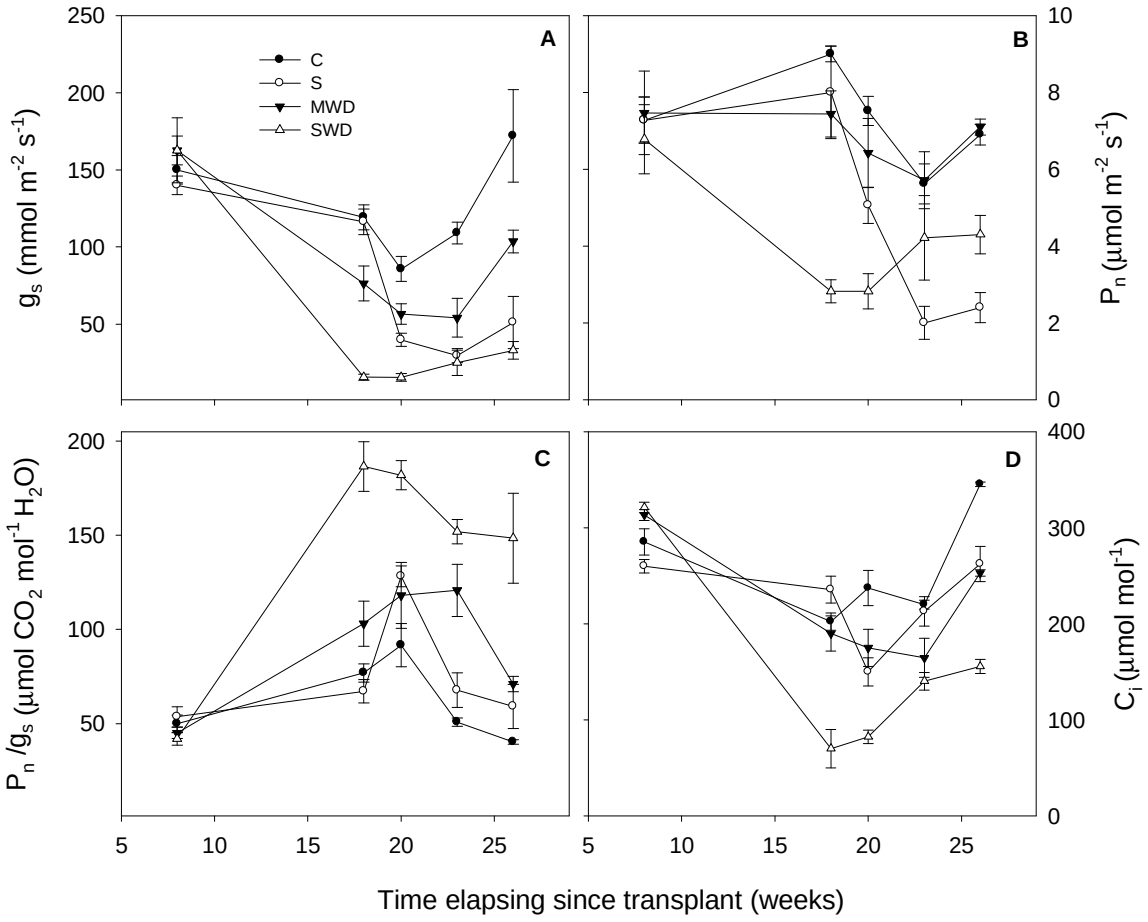

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623

624 **Fig. 5**. Evolution of stomatal conductance (g_s, A), net photosynthetic rate (P_n, B),
625 intrinsic water use efficiency (P_n/g_s, C) and internal CO_2 concentration (C_i, D) in *P.*
626 *purpurea* plants subjected to different irrigation treatments. Values are means of five
627 plants per treatments and the vertical bars indicate standard errors.